Hayati, Desember 2005, hlm. 131-138 Vol. 12, No. 4
ISSN 0854-8587

Photosynthesis of C3 and C4 Species in Response to Increased CO_2 Concentration and Drought Stress

HAMIM

Department of Biology, Bogor Agricultural University, Jalan Raya Pajajaran, Bogor 16144
Tel./Fax. +62-251-345011, E-mail: hamim@ipb.ac.id

Diterima 1 Februari 2005/Disetujui 7 Oktober 2005

Photosynthetic gas exchange in response to increased carbon dioxide concentration ($[CO_2]$) and drought stress of two C3 (wheat and kale) and two C4 species (*Echinochloa crusgallii* and *Amaranthus caudatus*) were analysed. Plants were grown in controlled growth chambers with ambient (350 µmol mol^{-1}) and doubled ambient $[CO_2]$. Drought was given by withholding water until the plants severely wilted, whereas the control plants were watered daily. Even though stomatal conductance (*Gs*) of C4 species either under ambient or double $[CO_2]$ was lower than those in C3, doubled $[CO_2]$ decreased *Gs* of all species under well watered conditions. As a result, the plants grown under doubled $[CO_2]$ transpired less water than those grown under ambient $[CO_2]$. Photosynthesis (*Pn*) of the C4 species was sustained during moderate drought when those of the C3 species decreased significantly. Doubled $[CO_2]$ increased photosynthesis of C3 but not of C4 species. Increased $[CO_2]$ was only able to delay *Pn* reduction of all species due to the drought, but not remove it completely. The positive effects of increased $[CO_2]$ during moderate drought and the disappearance of it under severe drought suggesting that metabolic effect may limit photosynthesis under severe drought.

INTRODUCTION

Increased $[CO_2]$ since the beginning of the 18th century has been postulated to influence climate causing a rise in atmospheric temperature and increased frequency of droughts around the world (IPCC 2001). This phenomena may have a significant effect on plant growth and species distribution around the world. The response of C3 species to increased $[CO_2]$ may be more positive than that of C4 species because photosynthetic rate of C3 species increases by approximately 58% due to doubled $[CO_2]$ (Drake *et al*. 1997). However, in C4 species, the photosynthesis is nearly saturated under recent ambient $[CO_2]$ (von Caemmerer *et al*. 1997). On the other hand, in conditions of higher temperature and drought, C4 species have been predicted to be more favoured than C3 species (Long 1999). This is due to the CO_2 concentrating mechanism (CCM) in C4 species enables the plants to maintain CO_2 assimilation rate when stomatal conductance is lower in limited water availability (Knapp & Medina 1999).

It has been suggested that increased $[CO_2]$ will increase water use efficiency (WUE) of C3 species, because it causes a reduction in transpiration rate and an increase in CO_2 assimilation rate of the plants. In C4 species, the positive effect of increased $[CO_2]$ on photosynthesis may be pronounced under drought conditions (Seneweera *et al*. 1998). However, a few recent papers have reported that elevated $[CO_2]$ has increased the growth of several C4 grasses even under well watered conditions (e.g. Wand *et al*. 1999; Ghannoum *et al*. 2000). The fertilizer effects of elevated $[CO_2]$ on C4 species is not well understood, even though evidence indicates that CO_2 enrichment increased leaf *Pn* (LeCain & Morgan 1998; Ziska *et al*. 1999), and reduced transpiration rate may cause an increase in *Pn* of C4 species by increasing leaf temperature (Ghannoum *et al*. 2000).

Increased $[CO_2]$ has also been predicted to have a positive effect on plants grown under drought stress because CO_2 enrichment may result in an increase of osmotic adjustment (OA) (Pospisilova & Catsky 1999). Increase of photosynthesis due to elevated [CO2] especially during the beginning of drought may improve solute accumulation such as sugars and organic acids required for osmotic adjustment (Pospisilova & Catsky 1999). Studies in poplar and willow (Johnson *et al*. 2002), *Betula papyrifera* (Kubiske & Pregitzer 1997) and *Quercus robur* (Picon *et al*. 1997) indicated an increase of OA due to double $[CO_2]$. These studies have suggested that C3 species may obtain more benefits of CO_2 enrichment under drought stress, even though under more frequent and severe drought C4 species have been predicted to achieve benefit more than C3 species (Ward *et al*. 1999). However, how far C3 and C4 species gain advantages of increased $[CO_2]$ under drought stress has not been resolved. Therefore, the investigation of the response of C3 and C4 species to increased $[CO_2]$ either under well-watered or water-stressed conditions is still needed. This experiment aims to understand the response of photosynthetic gas exchange of C3 (wheat and kale) and C4 (*Echinochloa crusgallii* and *Amaranthus caudatus*) species to the increased $[CO_2]$ under drought stress and to analyze drought stress amelioration due to high CO_2 in these types of species.

MATERIALS AND METHODS

The species used in this experiment were spring wheat (*Triticum aestivum* var. IMP) and kale (*Brassica oleraceae* L. var. Kestrel) for C3 species and *Echinochloa crusgallii* and *Amaranthus caudatus* for C4 species. The seeds were sown in a cabinet using a mixed medium of compost and perlite (1:1 v/v) to germinate over 1-2 weeks. The seedlings were

then transplanted to 17 cm diameter (2 L) pots using the same medium that was used for germination. The pots were then placed in the controlled cabinet (Fitotron Model number SGC660/C/HQI, Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK). The CO_2 concentration in the cabinet was maintained at ambient (350 µmol mol^{1}) and doubled CO_2 concentration (700 µmol mol^{1}) which was measured continuously with a gas monitor (WMA-2, PP Systems, Hitchin Herts, UK) controlled automatically by a computer. For every species, 64 pots of the plants were grown in two cabinets (32 pots each for ambient and double ambient [CO_2]).

Drought Treatment and Watering. Four weeks after planting, the drought was given by withholding water until the plants severely wilted, whereas the control plants were watered daily. During the drought treatment, fertilizer was neither given to the control nor to the stressed plants. After the time of stress the plants were rewatered as the control plants to assess their recovery. Water consumption was monitored by weighing the pots daily. Water availability was maintained at approximately the same amount by adding water to any pots whose water content fell below the average amount for all other pots. Water monitoring was carried out by weighing pots everyday. If the weight was lower than the average weight, water were added through plastic tubes installed on the pot with different depth.

Water Status Measurement. Plant water status was analysed during the treatment by measuring media water content, leaf relative water content, leaf water potential and leaf osmotic potential. Media water content was measured on a fresh weight basis by drying the media samples in the 80 °C oven for 3 days. To determine leaf relative water content (RWC), the disks or sections of the leaves were weighed (FW), and then floated on water for 16-24 h at the temperature 4 °C in the dark to get the tissues fully hydrated (HW). After weighing, the hydrated disks were dried in an 80 °C oven for 3 days to get dry weight (DW). Relative water content (RWC) was determined as followed: RWC = ((FW - DW)/(HW - DW)) x 100%, where: FW is fresh weight, DW is dry weight of the leaf, and HW is hydrated weight.

Leaf water potential (WP) was measured with pressure chamber (SKPM 1400, Skye Instruments Ltd, Powys, UK). Osmotic potential (OP) of leaf sap was estimated by a thermocouple psychrometer, (Tru-psy SC10X, Decagon Devices, Inc., Pullman, Washington). Every measurement, the sample was repeated with three replications per treatment taken from randomised three pots.

Gas Exchange Measurement. Net CO_2 assimilation (*Pn*) and stomatal conductance (G_s) were measured on the youngest fully expanded leaf (at least 5 samples per treatment) using a portable leaf chamber and gas exchange analyzer (CIRAS 1 Combined Infrared Gas Analysis System, PP System, Hitchin Herts, UK). Measurements were conducted with the temperature between 19-21 °C. The conditions used during measurement (the reference in the chamber) were 350 µmol mol^{-1} CO_2 for ambient and 700 µmol mol^{-1} CO_2 for increased CO_2 plants, a RH of about 70% corresponding to a 1.20 kPa leaf air vapour pressure deficit (VPD). *Pn* were measured under 450 µmol photons m^{-2} s^{-1} provided by the cabinet light (Powerstar HQI-TS 250W, Osram, Hamburg, Germany).

RESULTS

Elevated [CO_2] Increased Plant Water Status. Media water content (MWC) of all species either under ambient or doubled [CO_2] decreased gradually due to drought treatment. During the treatment, kale was wilted after 9 days of drought followed by *Amaranthus* and wheat (after 11 and 12 days), while *E. crusgallii* was wilted after 15 days drought. Drought treatment decreased MWC of wheat, kale and *A. caudatus* to 20-25%, while it only decreased that of *E. crusgallii* to approximately 40% (Table 1). Plants grown under ambient [CO_2] transpired more water than those grown under increased [CO_2] during the drought treatment based on the data water lost monitored daily by weighing the pots (data not shown). However, because MWC of the plants in both cabinets was maintained at approximately the same value by adding some amount of water, there was no difference in reduction of MWC between plants in ambient and doubled [CO_2] during the drought (Table 1).

In well watered conditions double [CO_2] did not affect water status of all species as indicated by the value of leaf relative water content (RWC), water potential (WP) and osmotic potential (OP) (Table 1). However, under drought stress, the C3 species (wheat and kale) have lower RWC, WP and OP at the end of the drought cycle than C4 species *E. crusgallii* and *A. caudatus* (Table 1) Double [CO_2] did not affect RWC of wheat and and *A. caudatus* during drought stress, but it caused an increase in RWC of kale and *E. crusgallii* compared to the RWC of those on ambient [CO_2] (Table 1).

Table 1. Media water content (MWC), relative water content (RWC), water potential (WP) and osmotic potential (OP) of wheat (Wh), kale (Kl), *E. crusgallii* (Ec) and *A. caudatus* (Ac) grown in ambient (A) and elevated [CO_2] (E) under well watered (C) and drought stressed (S) condition. SE indicates standard error of the data.

Species	Treatments	Parameters			
		MWC	RWC	WP	OP
		..... (%)		..... (MPa)	
Wh	AC	64.1	92.7	-0.27	-1.11
	AS	22.2	50.2	-3.11	-2.72
	EC	65.9	93.6	-0.25	-1.11
	ES	22.0	52.6	-2.61	-2.23
	SE	(0.60)	(4.08)	(0.16)	(0.11)
Kl	AC	56.2	94.3	-0.37	-1.09
	AS	21.7	56.4	-2.14	-1.82
	EC	58.5	92.0	-0.51	-1.08
	ES	23.1	74.3	-1.71	-1.61
	SE	(1.76)	(3.78)	(0.05)	(0.06)
Ec	AC	62.5	93.1	-0.40	-0.88
	AS	40.7	74.7	-1.53	-1.29
	EC	62.2	96.2	-0.37	-0.81
	ES	42.0	82.2	-1.27	-1.18
	SE	(1.16)	(2.87)	(0.03)	(0.05)
Ac	AC	56.2	95.6	-0.35	-0.89
	AS	26.1	79.0	-1.48	-1.31
	EC	56.5	96.5	-0.34	-0.90
	ES	24.7	79.8	-1.43	-1.15
	SE	(1.37)	(4.98)	(0.05)	(0.09)

Effect of Increased [CO_2] and Drought Stress on Stomatal Conductance, Net CO_2 Assimilation and Intercellular CO_2 Concentration. The effects of CO_2 concentration and drought stress on gas exchange parameters varied between species. In well-watered conditions double [CO_2] reduced stomatal conductance (*Gs*) of wheat, kale and *E. crusgallii* by approximately 20-30%, but in *A. caudatus* it did not (Figure 1). In the C3 species, *Gs* decreased rapidly from the beginning until moderate drought, when *Gs* of plants grown in both ambient and elevated [CO_2] showed similar values. Thereafter, *Gs* reduction continued more slowly until the end of the drought. In the C4 species, *Gs* reduction was almost linear from the beginning until the end of the drought. At the end of the drought, *Gs* of all species dropped to near zero, except in *E. crusgallii* where the stomata remained open and *Gs* was approximately 60 mmol m^{-2} s^{-1}. Two days after rewatering *Gs* was completely restored except in *A. caudatus* grown under ambient [CO_2] which only partially restored (Figure 1). In severe drought *Gs* of all species grown in double [CO_2] was similar to that of the plants grown in ambient [CO_2].

Photosynthetic CO_2 assimilation rate (*Pn*) of the C3 and C4 species responded differently to [CO_2]. When well-watered, *Pn* of the C3 species increased by approximately 15-25% due to doubled [CO_2], but there was no increase in the C4 species. Drought stress decreased *Pn* of all species gradually in both CO_2 treatments. During drought, *Pn* reduction in C3 species occurred soon after the drought progressed, while in C4 species it occurred later after moderate stress developed (Figure 2). Severe drought caused the *Pn* of all species to drop to near zero except in *E. crusgallii* where the *Pn* was still approximately 5 µmol m^{-2} s^{-1} even though the plants were extremely wilted. When growing in increased [CO_2], the *Pn* reduction due to drought stress was delayed for a few days in wheat and *E. crusgallii* but not in kale and *A. caudatus*. However, at the end of the drought, *Pn* of wheat and *E. crusgallii* grown in doubled CO_2 dropped by the same amount as that of the plants grown in ambient [CO_2], while in kale and *A. caudatus*, *Pn* of the plants grown in doubled [CO_2] was higher than that in ambient [CO_2] (Figure 2). Two days after rewatering the *Pn* of wheat and kale were completely restored, whereas *Pn* of *E. crusgallii* and *A. caudatus* were only partially restored.

Intercellular CO_2 concentration (*Ci*) differed between species under both ambient and elevated [CO_2]. In ambient [CO_2], *Ci* of well-watered C3 species was approximately 250 µmol mol^{-1} while in the C4 species *Ci* was between 150-200 µmol mol^{-1} (Figure 3). Doubled [CO_2] increased *Ci* of C3 species to 500-520 µmol mol^{-1}, and of C4 species to 440-500 µmol mol^{-1}. Drought stress caused a decrease in *Ci* of wheat, kale and *A. caudatus* grown either in ambient or in doubled [CO_2] but no similar reduction was observed for *E. crusgallii*. However, reduction of the *Ci* due to drought stress occurred only until moderate drought, and when the drought became severe the *Ci* increased and became higher than that of control plants, except in *A. caudatus* grown in elevated [CO_2] where the *Ci* reduced simultaneously until the end the drought cycle (Figure 3). In *E. crusgallii* grown in doubled [CO_2], *Ci* also increased during the last period of the drought cycle. Two days after rewatering, *Ci* of all species was restored completely to that of control plants.

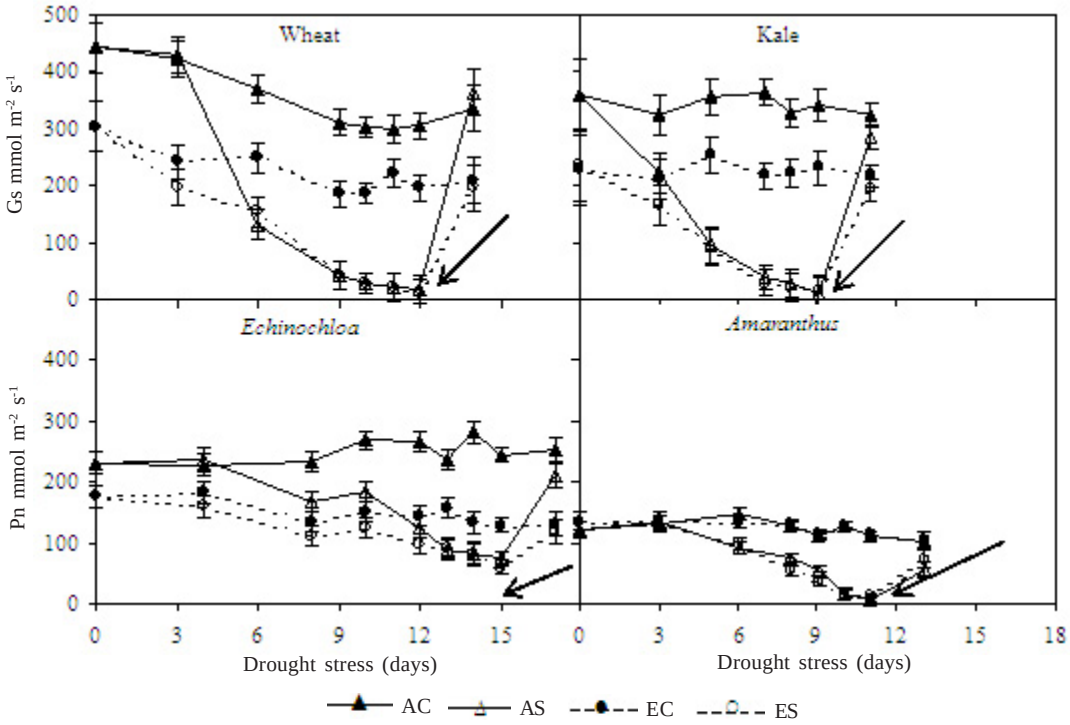

Figure 1. Stomatal conductance (*Gs*) of well watered (C) and drought stressed (S) wheat, kale, *E. crusgallii* and *A. caudatus* grown in ambient (A) and double CO_2 concentration (E) during drought stress and rewatering. The arrow indicates time of rewatering.

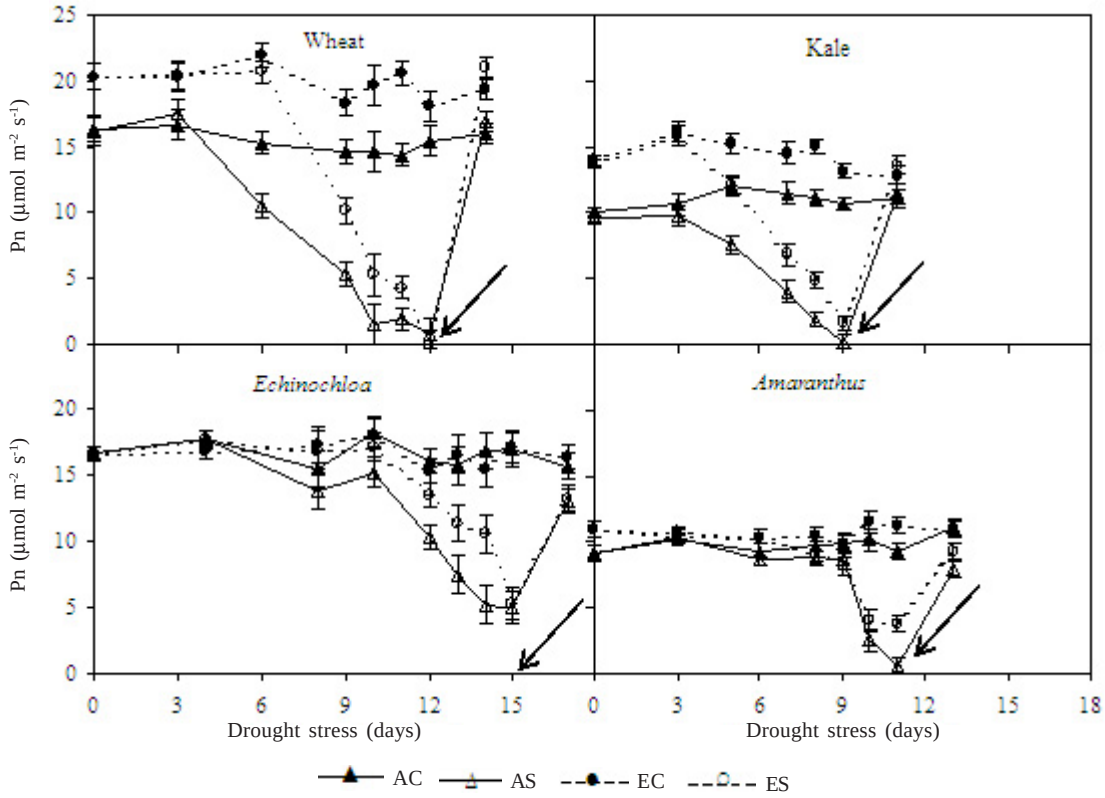

Figure 2. Net CO_2 assimilation rate (Pn) of well watered (C) and drought stressed (S) wheat, kale, *E. crusgallii* and *A. caudatus* grown in ambient (A) and double CO_2 concentration (E) during drought stress and rewatering. The arrow indicates time of rewatering.

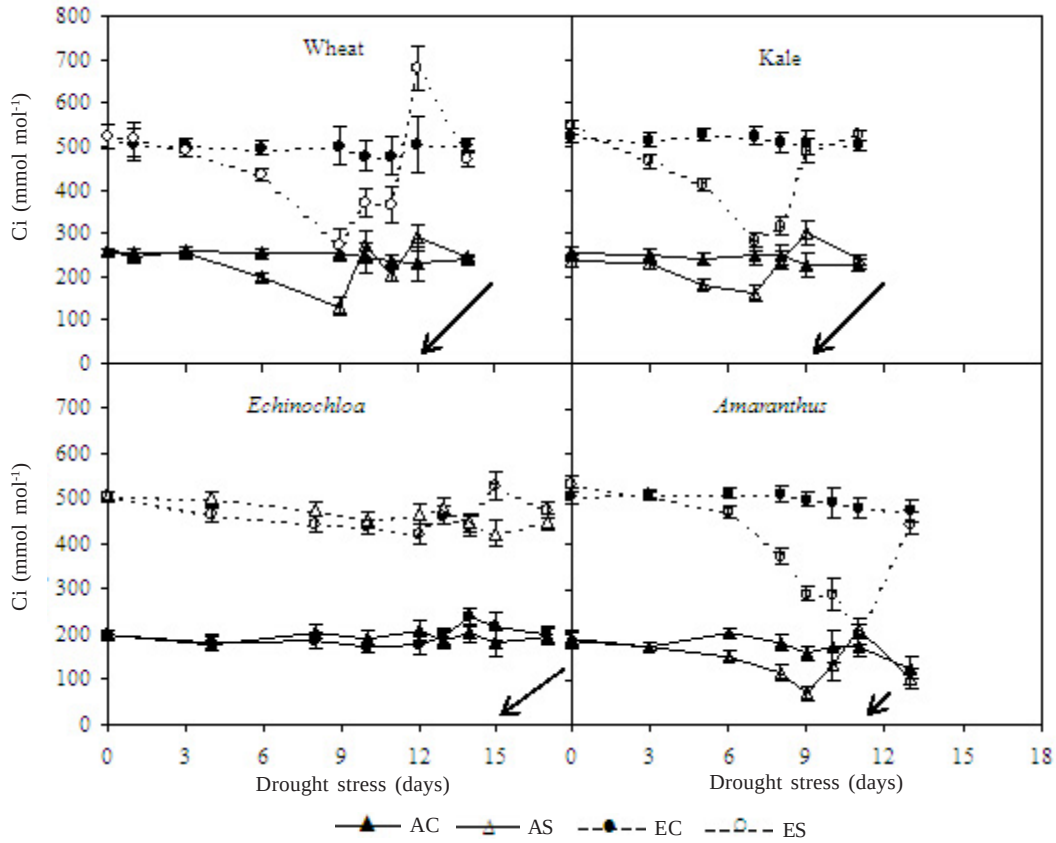

Figure 3. Intercellular CO_2 concentration (*Ci*) of well watered (C) and drought stressed (S) wheat, kale, *E. crusgallii* and *A. caudatus* grown in ambient (A) and double CO_2 concentration (E) during drought stress and rewatering. The arrow indicates time of rewatering.

Response of Stomatal Conductance and Photosynthesis to Plant Water Status. The time course and sensitivity to drought varied between species and CO_2 treatment. In order to compare them, the relationship of the relative stomatal conductance (R*Gs*) and relative net CO_2 assimilation rate (R*Pn*) to the WP was examined. The curve of R*Gs* of all species was exponential with *Gs* reduced markedly at slight reductions of WP (Figure 4). The water potential at maximum *Gs*, WPM_{Gs} was between -0.2 and -0.4 MPa with wheat the highest and *E. crusgallii* the lowest WPM_{Gs} respectively. However, the $\Delta RGs/\Delta WP$ of wheat was the largest followed by kale and *A. caudatus*, while that of *E. crusgallii* was the smallest (Table 2). Doubled CO_2 caused a decrease in WPM_{Gs} and $\Delta PGs/\Delta WP$ of wheat, kale and *E. crusgallii*, but not of *A. caudatus* (Table 2).

The response of relative net CO_2 assimilation rate, R*Pn*, to reduced WP was different between C3 and C4 species and growth [CO_2] (Figure 5). In C3 species, the curves were exponential with slight reductions of WP (from -0.2 to -1.0 MPa), causing a marked *Pn* reduction. On the other hand, in the C4 species the curves were quadratic with less *Pn* reduction when WP > -1.0 MPa (more than -1 MPa) (Figure 5). Therefore, the change of the R*Pn* to WP curve ($\Delta RPn/\Delta WP$) was lower in C4 species than in C3 species (Table 3).

DISCUSSION

Stomatal Conductance Sensitivity to Drought Stress and [CO_2] was Different Between C3 and C4 Species. Stomatal conductance (*Gs*) of well-watered *E. crusgallii* and *A. caudatus* either under present ambient or double [CO_2] was lower than that in wheat and kale respectively (Figure 1). This was consistent with the general characteristic of C4 species which have lower *Gs* than C3 species under the same atmospheric conditions (Long 1999; Knapp & Medina 1999). Drought stress caused a dramatic reduction of *Gs* in the C3 species especially during the beginning of the drought while in the

Table 2. The change of relative stomatal conductance (R*Gs*) with WP reduction ($\Delta RGs/\Delta WP$) of plants grown in ambient and doubled [CO_2]. The data were analysed from the curve stated in Figure 5 between the WP where R*Gs* was 100% (WPM_{Gs}) and the WP was 0.5 MPa lower than WPM_{Pn}.

Species	Ambient CO_2		Increased CO_2	
	WPM_{Gs} (MPa)	$\Delta RGs/\Delta WP$ (MPa^{-1})	WPM_{Gs} (MPa)	$\Delta RGs/\Delta WP$ (MPa^{-1})
Wheat	-0.203	1.866	-0.303	1.546
Kale	-0.302	1.548	-0.351	1.322
E. crusgallii	-0.403	0.940	-0.418	0.667
A. caudatus	-0.236	1.000	-0.273	1.061

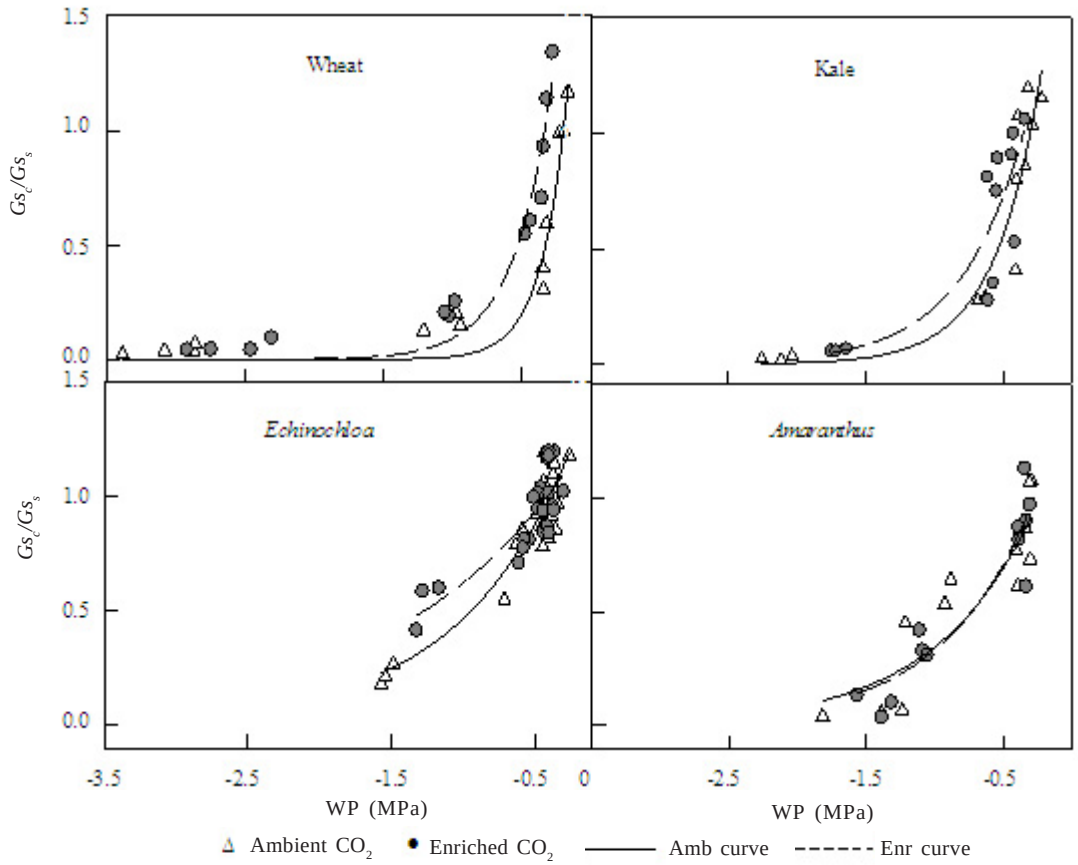

Figure 4. Relative stomatal conductance (R*Gs*) of wheat, kale, *E. crusgallii* and *A. caudatus* grown in ambient and increased [CO_2] in response to reduced water potential (WP) due to drought stress. Equation of the form is R*Gs*=a*exp(b*WP).

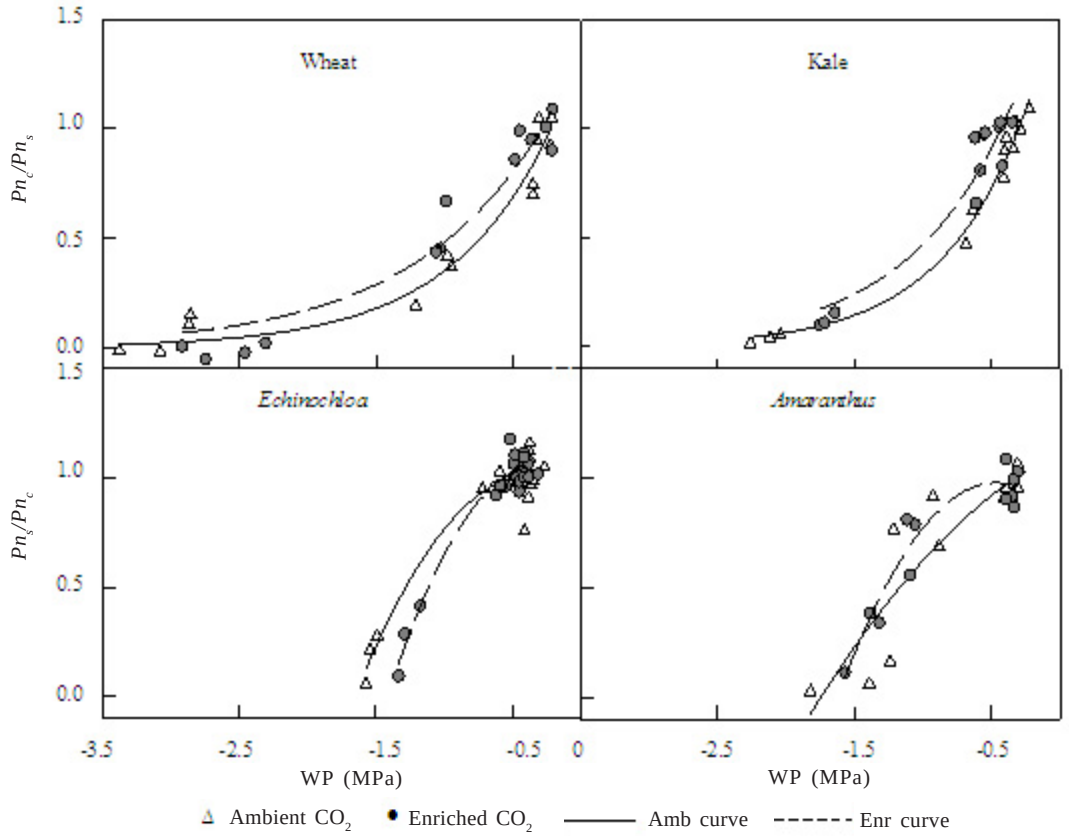

Figure 5. Relative net CO_2 assimilation rate (R*Pn*) of wheat, kale, *E. crusgallii* and *A. caudatus* grown in ambient and increased [CO_2] in response to reduced water potential (WP) due to drought stress. Equation of the form is R*Pn*=a*exp(b*WP) for wheat and kale and *Pn*=y0+a*WP+b*WP^2 for *E. crusgallii* and *A. caudatus*.

Table 3. The change of the curve of relative assimilation rate (R*Pn*) response to WP (DR*Pn*/DWP) of plants grown in ambient and doubled [CO_2]. The data were analysed from the curve (Figure 6) between the WP where R*Pn* was 100% (WPM_{Pn}) and the WP was 0.5 MPa lower than WPM_{Pn}.

Species	Ambient CO_2		Increased CO_2	
	WPM_{Pn} (MPa)	ΔR*Pn*/ΔWP (MPa^{-1})	WPM_{Pn} (MPa)	ΔR*Pn*/ΔWP (MPa^{-1})
Wheat	-0.217	0.977	-0.285	0.801
Kale	-0.299	1.108	-0.426	0.971
E. crusgallii	-0.176	0.095	-0.086	0.021
A. caudatus	-0.330	0.525	-0.480	0.381

C4 species *Gs* decreased gradually during the progressive drought (Figure 1), and this was also evident from the response curves of *Gs* to WP (Figure 4).

To characterize stomatal sensitivity, the change of relative stomatal conductance (R*Gs*) to water potential (ΔR*Gs*/ΔWP) was analysed over -0.5 MPa from WP at maximum *Gs* determined from the curve at the Figure 4 (Table 2). The stomatal sensitivity (ΔR*Gs*/ΔWP) of these curves are 50-80% higher in C3 than in C4 species (Table 2).

Unlike in wheat, kale and *E. crusgallii* where the *Gs* decreased in increased [CO_2], the *Gs* in *A. caudatus* was not reduced and the response of *Gs* to WP did not change due to doubled [CO_2] (Figure 1 & Table 2). The reason for this insensitivity of *Gs* of *A. caudatus* to CO_2 may be because the low light intensity (approximately 450 µmol photons m^{-2} s^{-1}) as indicated by the low value of *Gs* even under well watered conditions (Figure 1). Several previous experiments have also shown insensitivity of conductance to increased [CO_2] e.g. sunflower (Huxman *et al*. 1999) and C4 grass *Bouteloua gracilis* (Morgan *et al*. 1998) but there were no specific explanations on this insensitivity. A common explanation about difference of stomatal sensitivity to high [CO_2] is due to the variation of growth conditions such as temperature, humidity and light (Morison 1985).

Doubled [CO_2] also reduced the ΔR*Gs*/ΔWP except in *A. caudatus* (Figure 4 & Table 2). The maximum WP, WPM_{Gs} of wheat, kale and *E. crusgallii* were also decreased in doubled [CO_2], suggesting that increased [CO_2] postponed the start of *Gs* reduction. These results suggest that doubled [CO_2] reduced stomatal conductance sensitivity to drought stress of wheat, kale and *E. crusgallii*.

Different Response of Photosynthetic Gas Exchange to Drought Stress Between C3 and C4 Species in Different [CO_2]. Drought stress caused a substantial reduction of *Pn* and at the end of the drought cycle *Pn* of the C3 species grown in either ambient or doubled [CO_2] was almost zero (Figure 2). Reduced *Pn* during mild drought stress was associated with a decrease of intercellular CO_2 concentration, *Ci* (Figure 3), suggesting that stomatal closure limits photosynthesis in the C3 species. However, when drought

became severe the reduced *Pn* was not related to low *Ci* because *Ci* even increased more than that of well watered plants (Figure 3) indicating that there were nonstomatal effects on photosynthesis (Berkowitz 1998; Flexas & Medrano 2002).

In the C4 species *Pn* was sustained until moderate drought (9 days after drought began) even though the *Gs* of these species had reduced 6 days after drought began (Figure 1). In addition, the response curve of *Pn* to WP was different between the C3 and C4 species (Figure 5), with the C3 species showing very much higher gradient ($\Delta R Pn/\Delta WP$) than the C4 (Table 3). Clearly reduced stomatal conductance during the early stages of drought caused higher *Pn* reduction of C3 than C4 species, which is consistent with the concept of the CO_2 concentrating mechanism (CCM) of C4 species which enables the plant to maintain photosynthetic capacity under environmental stresses such as drought (Matsuoka *et al*. 2001). Because PEPCase in C4 photosynthesis is saturated at a much lower [CO_2] than Rubisco, any decrease in *Gs* will decrease *Pn* in a C3 species, whereas in C4 species it will only affect *Pn* if intercellular CO_2 is lowered below 100-150 µmol mol^{-1} (Long 1999).

Increased [CO_2] was only able to delay *Pn* reduction due to the drought, not remove it completely. Consequently, under severe drought the positive effect of doubled [CO_2] on *Pn* of C3 species disappeared (Figure 2). Since the media water content of plants grown in both CO_2 treatments was maintained in the same condition, the postponement of *Pn* reduction under increased [CO_2] was mainly due to the positive effects of CO_2 enrichment on *Pn* when stomata limited photosynthesis. In addition, during moderate drought, *Gs* of the C3 species in both CO_2 treatments was similar (Figure 1). The disappearance of positive effects of increased [CO_2] under severe drought suggested that metabolic effects limited photosynthesis. These results are consistent with previous reports that increased CO_2 can ameliorate the *Pn* of some C3 species in moderate drought but not in severe drought such as in *Quercus robur* (Picon *et al*. 1997), *Eucalyptus cladocalyx* (Palanisamy 1999), cherry (Centritto *et al*. 1999), and poplar and willow (Johnson *et al*. 2002).

Even though it had no effect under well-watered conditions, growth in increased [CO_2] reduced the drop in *Pn* of C4 species under drought stress (Figure 2). In *A. caudatus*, at the end of the drought cycle when the *Pn* dropped almost to zero, the plants grown in doubled [CO_2] still had *Pn* of about 35% of that of controls. This result is in agreement with another experiment using the C4 grass *Panicum coloratum* indicating that the positive effect of increased [CO_2] was more pronounced under drought than under well-watered conditions (Seneweera *et al*. 1998). The response curve of relative *Pn* to WP (Figure 5) in high [CO_2] grown plants showed a reduced $\Delta R Pn/\Delta WP$ in both the C3 and C4 species (Table 3) showing that increased [CO_2] partially ameliorated *Pn* reduction.

ACKNOWLEDGMENTS

I am grateful to QUE-Project of Department of Biology (1997-2002), Faculty of Mathematics and Natural Sciences, Bogor Agricultural University (IPB) for funding this research program. I also would like to thank James I.L. Morison (Department of Biological Sciences, University of Essex, Colchester, United Kingdom) for his valuable advise and help.

REFERENCES

Berkowitz GA. 1998. Water and salt stress. In: Raghavendra AS (ed). *Photosynthesis: A Comprehensive Treatise*. Cambridge: Cambridge Univ Pr. p 226-237.

Centritto M, Magnani F, Lee HSJ, Jarvis PG. 1999. Interactive effects of elevated [CO_2] and drought on cherry (*Prunus avium*) seedlings. II. Photosynthetic capacity and water relations. *New Phytol* 141:141-153.

Drake BG, Gonzalez-Meler MA, Long SP. 1997. More efficient plants: A consequence of rising atmospheric CO_2? *Ann Rev Plant Physiol Plant Mol Biol* 48:609-639.

Flexas J, Medrano H. 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals Bot* 89:183-202.

Ghannoum O, von Caemmerer S, Ziska LH, Conroy JP. 2000. The growth response of C_4 plants to rising atmospheric CO_2 partial pressure: a reassessment. *Plant Cell Environ* 23:931-942.

Huxman KA, Smith SD, Neuman DS. 1999. Root hydraulic conductivity of *Larrea tridentata* and *Helianthus annuus* under elevated CO_2. *Plant Cell Environ* 22:325-330.

[IPCC] Intergovernmental Panel on Climate Change. 2001. Summary for policymakers. In: Houghton JT *et al*. (ed). *Climate Change 2001: The Scientific Basic*. Cambridge: Cambridge Univ Pr. p 1-20.

Johnson JD, Tognetti R, Paris P. 2002. Water relation and gas exchange in poplar and willow under water stress and elevated CO_2. *Physiol Plant* 115:93-100.

Knapp AK, Medina E. 1999. Success of C4 photosynthesis in the field: lessons from communities dominated by C4 plants. In: Sage RF, Monson RK (ed). *C4 Plant Biology*. London: Academic Pr. p 251-283.

Kubiske ME, Pregitzer KS. 1997. Ecophysiological responses to simulated canopy gaps of two tree species of contrasting shade tolerance in elevated CO_2. *Funct Ecol* 11:24-32.

LeCain DR, Morgan JA. 1998. Growth, gas Exchange, leaf nitrogen and carbohydrate concentration in NAD-ME and NADP-ME C_4 grasses grown in elevated CO_2. *Physiol Plant* 102:297-306.

Long SP. 1999. Environmental responses. In: Sage RF, Monson RK (ed). *C4 Plant Biology*. London: Academic Pr. p 215-249.

Matsuoka M, Furbank RT, Fukayama H, Miyao M. 2001. Molecular engineering of C4 photosynthesis. *Ann Rev Plant Physiol Plant Mol Biol* 52:297-314.

Morgan JA, LeCain DR, Read JJ, Hunt HW, Knight WG. 1998. Photosynthetic pathway and ontogeny affect water relations and the impact of CO_2 on *Bouteloua gracilis* (C4) and *Pascopyrum smithii* (C3). *Oecologia* 114:483-493.

Morison JIL. 1985. Sensitivity of stomata and water use efficiency to high CO_2. *Plant Cell Environ* 8:467-474.

Palanisamy K. 1999. Interactions of elevated CO_2 concentration and drought stress on photosynthesis in *Eucalyptus cladocalyx* F-Muell. *Photosynthetica* 36:635-638.

Picon C, Ferhi A, Guehl JM. 1997. Concentration and $\delta^{13}C$ of leaf carbohydrates in relation to gas exchange in *Quercus robur* under elevated CO_2 and drought. *J Exp Bot* 48:1547-1556.

Pospisilova J, Catsky J. 1999. Development of water stress under increased atmospheric CO_2 concentration. *Biol Plant* 42:1-24.

Seneweera SP, Ghannoum O, Conroy J. 1998. High vapour pressure deficit and low soil water availability enhance shoot growth responses of a C4 grass (*Panicum coloratum* cv. Bambatsi) to CO_2 enrichment. *Aust J Plant Physiol* 25:287-292.

von Caemmerer S *et al*. 1997. Carbon isotope discrimination during C4 photosynthesis: insights from transgenic plants. *Aust J Plant Physiol* 24:487-493.

Wand SJE, Midgley GF, Jones MH, Curtis PS. 1999. Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a test of current theories and perceptions. *Glob Change Biol* 5:723-741.

Ward JK, Tissue DT, Thomas RB, Strain BR. 1999. Comparative responses of model C3 and C4 plants to drought in low and elevated CO_2. *Glob Change Biol* 5:857-867.

Ziska LH, Sicher RC, Bunce JA. 1999. The impact of elevated carbon dioxide on the growth and gas exchange of three C4 species differing in CO_2 leak rates. *Physiol Plant* 105:74-80.